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EXPERIMENTAL INVESTIGATIONS REGARDING THE ETIOLOGY OF DENGUE FEVER.*

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INTRODUCTION.

HAVING been instructed by the surgeon-general of the army to investigate the cause of dengue and to determine the possibility of the transmission of the disease by mosquitoes, we undertook the experiments detailed below when the opportunity was afforded by the occurrence of the disease in epidemic form at Fort William McKinley, Rizal, Luzon, P. I., four miles out of Manila. At this post, during the months of July, August, September, and October, there occurred over 800 cases of dengue, 128 of which were transferred to our dengue hospital in Manila for observation and experiment.

ETIOLOGY.

The etiological factor concerned in the causation of dengue has been sought for by almost every investigator who has studied the disease; thus, nearly every fluid, secretion, and excretion of the body has been examined, and, as might be expected, not a few observers have announced the discovery of the causative organism. Many of these so-called "discoveries" were made during the beginning of bacteriological science, and a perusal of the methods adopted in isolating the organisms described, as well as the description of their morphology, is sufficient to prove their absolute lack of scientific accuracy. We have not, therefore, deemed it necessary to review such contributions; but among the many who have investigated the etiology of this disease there are a few whose observations demand consideration. Among these may be mentioned McLaughlin, Graham, Carpenter and Sutton, Guiteras and Cartaya, and Agramonte.

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The early investigations regarding the etiology of dengue were directed toward a bacterial cause, but within the past few years the increasing importance of protozoa in the etiology of disease has turned the current of investigation, and almost all of the work that has been done upon this subject by recent investigators has been founded upon the belief that the disease is caused by some protozoon, probably by one infecting the blood.

The earliest attempt, based upon bacteriological methods, to discover the organism causing dengue was that of McLaughlin.[1] The descriptions of his experiments are detailed and the results obtained of interest, but, viewed in the light of the approved bacteriological methods of today, are open to very severe criticism and have not been confirmed by later observers.

McLaughlin examined both fresh and stained specimens of blood, made cultures from blood upon nutrient gelatin and also endeavored to secure cultures by partially filling sterilized glass bulbs with blood from a vein and incubating for weeks and even months. In the blood of every case upon microscopical examination he found micrococci, red or purplish in color, and in cultures upon nutrient gelatin, the same cocci, appearing black or brown when in masses, but when seen singly the red color was always distinct and characteristic. In one case, in the bulbs mentioned, which contained blood but no nutrient material, he found the same organism after an incubation period of from six weeks to three months.

While the researches of McLaughlin appear to have been partially accepted, or, at least, considered seriously, by some writers, we regard them as of purely historical interest, for reasons which are obvious.

The observations of Graham[2] of Beirut, published in 1903, renewed interest in the etiology of this disease, and a considerable amount of work has since been done with the object of confirming or disproving his results. He announced, as the result of his work, that dengue is caused by a protozoon, inhabiting the red-blood corpuscles, and closely resembling the plasmodia of malaria, except for the absence of pigment. He was not able to demonstrate the organism in stained specimens of blood, nor did he observe any sporulating forms. He considered that the organism underwent a developmental stage within the mosquito, and claimed to have demonstrated it in the cells of the salivary glands in mosquitoes of the genus *Culex fatigans*, but he was not able to observe any *zygotes* or any evidence of sexual forms within the mosquito.

Graham also details in his report the results of certain experiments which he made and in which he claims to have proven that dengue is transmitted by the mosquito. Of six healthy men bitten by infected mosquitoes (*Culex fatigans*) under circumstances which appear to have obviated all chance of infection in other ways, five developed dengue, two in four days, two in five days, and one in six days after having been bitten.

Because of the positive character of Graham's statements, his work attracted widespread attention, but though many experienced microscopists have endeavored to do so, none have been able to confirm his results as regards the presence of a parasite in the blood; but we believe that, however much he may have erred in his interpretation of the bodies described by him in the blood, his experiments regarding the method of transmission are most valuable, and his conclusion that dengue is transmitted by the mosquito is well founded and has been experimentally confirmed by us.

Upon the Isthmus of Panama, Carpenter and Sutton[3] studied 200 cases of dengue, examining both fresh and stained specimens of blood, but were unable to demonstrate either McLaughlin's micrococcus or Graham's protozoon. They also undertook some experiments with mosquitoes but did not obtain any results which they considered of importance.

Guiteras and Cartaya,[4] as the result of a very careful investigation carried on in Havana in 1905, believe that Graham is mistaken regarding his organism, and conclude, after examining a large series of blood specimens, taken during all days of the disease, at various hours, and stained by various methods, that the blood contains no structure resembling a parasite. They also endeavored to transmit the disease by mosquitoes, but with negative results. Guiteras states, regarding these experiments, that their small number and lack of variety deprive the negative result of a claim to conclusive character, and that their faith remains unshaken that the mosquito is the transmitter of dengue.

Agramonte,[5] studying the disease in Havana in 1906, was unable to demonstrate any parasite in the blood, nor was he able to transmit the disease by the mosquito. He states, however, that he believes that the mosquito transmits the disease and that his negative results were due to some undiscovered fault in technique.

The recent researches of Kieweit de Jonge and de Haan in Java and of Stitt[6] in Manila were without result as regards the presence in the blood of any parasite of etiological importance.

EXAMINATION OF BLOOD.

In attempting to solve the etiology of dengue and its method of transmission, our attention was first directed to the microscopical examination of the blood of patients suffering from the disease. Despite the failure of others to demonstrate any parasite in the blood, we considered that our work would be incomplete without careful examination of both fresh and stained preparations of the blood, and accordingly we have studied thoroughly, in this respect, a large number of our cases; the blood was examined during every period of the disease, but especially during the first two days and during the terminal rise in the temperature; various staining methods were used, including Wright's stain and the methods used in demonstrating *Treponema pallidum*. The latter methods were used very carefully and in numerous cases, as at the time we began our work we were greatly inclined to believe that the organism causing dengue might belong to the *Spirochetae*. We have not been able to confirm the results of McLaughlin or Graham, nor have we been able to demonstrate any organism in the blood of dengue patients, which we can consider as the cause of the disease.

The following is a résumé of the changes observed by us as occurring in the blood in dengue.

Hemoglobin and color index.—In uncomplicated cases the hemoglobin and color index are normal.

The red corpuscles.—Dengue is not a disease in which anemia is present. We have made numerous blood counts in severe cases, and have never observed a count lower than 4,500,000 red cells per c.m., even when the count was made at the termination of the disease. This fact alone appears to us to disprove conclusively the existence of Graham's hematozoon, which, by destroying the red corpuscles during its development within them, would certainly reduce them in number. We have never seen a case of uncomplicated dengue in which the clinical symptoms suggested anemia.

Morphology of the red corpuscles: In size the red corpuscles are unchanged. Poikilocytosis is not commonly observed, but in some cases, during the height of the fever, a moderate degree of poikilocytosis may be present. Crenation does not occur more rapidly nor is it more marked in dengue than in other acute febrile conditions. Vacuolation is common both in fresh and stained specimens of dengue blood, and in many instances the shape and appearance of the vacuoles is very suggestive of a parasitic invasion of the red cell; artefacts, due to degeneration of the protoplasm and clear areas due to the retraction of the hemoglobin, are common, especially in poorly prepared smears, and are well calculated to lead to error because of resemblance to bacterial or protozoal organisms. We have not observed that the appearance of the vacuoles occurring in the red corpuscles in dengue differs from that observed in many other febrile conditions, but it is certainly true that they frequently present an appearance very suggestive of ameboid motion without change of position; the progressive motion referred to by some writers we have observed in the case of rod-shaped artefacts, probably due to protoplasmic currents within the degenerating red cell.

It is not uncommon to observe in the blood of dengue, as well as in that of other febrile conditions, cocci or bacilli, either free in the blood plasma or attached to the red-blood corpuscles. In the vast majority of instances these bacteria are due to external contamination and have no relation to the disease in which they are observed. When they are attached to the red corpuscle, and still possess some motility, their resemblance to an intracellular parasite is often striking, but it is

usually possible, by gentle pressure, to dislodge them and thus demonstrate their real nature. We have not observed the presence of normoblasts or megaloblasts in the blood in dengue, and the absence, especially of normoblasts, indicates that anemia, even of a mild type, is not present.

The staining reactions of the red corpuscles in dengue do not differ from those present in health. Polychromatophilia or basophilia we have not observed, but in poorly prepared specimens the staining may be irregular, suggesting granular degeneration.

The leucocytes.—Number: One of the most important blood changes in this disease is the presence, in almost every case, of a marked leucopenia. From our observations we are convinced that the leucopenia of dengue is almost constant throughout the attack, and that it is of considerable diagnostic importance. We have made leucocyte counts in a large number of cases and have invariably found marked reduction in the total number of leucocytes, with, as will be seen later, quite a characteristic change in the relative proportion of the various forms. The lowest leucocyte count we have seen was 1,200 per c.m., the highest, 4,860 per c.m. the average, 3,800 per c.m. We have found that the leucopenia is progressive, being most marked upon the fifth day of the disease, or, sometimes, upon the sixth.

Morphology: We have observed no morphological changes in the leucocytes, or any evidence of the presence of a leucocytozoon.

Differential leucocyte count: From the studies of Carpenter and Sutton, and later, of Stitt, the differential leucocyte count in dengue has assumed considerable diagnostic importance, and, taken together with the leucopenia, appears to us to be entitled to very careful consideration in the differential diagnosis of dengue, yellow fever, malaria, and the eruptive fevers. Carpenter and Sutton,[7] from their blood examinations, conclude that in dengue there is always a leucopenia, and generally an increase in the small lymphocytes and in the eosinophiles, the latter occurring late in the disease. Stitt[8] made differential leucocyte counts at varying periods of the disease. He found marked variation occurring in the number of the various forms of leucocytes at different periods, there being at first a great increase in the small lymphocytes, succeeded by a greater increase in the large

ymphocytes and mononuclears, and finally, during the terminal eruption, a most marked increase in the large mononuclears.

Because of lack of time we have made but comparatively few differential counts, but our results have been supplemented by those of Lieutenent Vedder, Medical Department, U. S. A., stationed at Fort William McKinley, who kindly volunteered to assist us in this direction. From our own observations we are loth to lay as much stress upon the variation of the relative proportions of the large and small lymphocytes, at varying stages of the disease, as does Stitt, for in many instances we have found that no constant relationship exists between the variety of lymphocyte increased and the period of the disease, but we have found a constant leucopenia, a decrease in the polymorphonuclears, and an increase in small lymphocytes at every stage. In one of our experimental cases in whom we produced a severe attack of dengue by the intravenous inoculation of filtered blood from another experimental case, the leucocyte count, made upon the first, third, and sixth day of the disease, well illustrates the changes described by Stitt, as is shown by the following record of the counts.

FIRST DAY OF DISEASE.

Polymorphonuclears	50.0%
Small lymphocytes	41.0
Large lymphocytes	7.5
Eosinophiles	1.5

THIRD DAY OF DISEASE.

Polymorphonuclears	52%
Small lymphocytes	36
Large lymphocytes	8
Eosinophiles	4

SIXTH DAY OF DISEASE.

Polymorphonuclears	48%
Small lymphocytes	14
Large lymphocytes	32
Eosinophiles	6

It will be observed that the eosinophiles increased as the disease progressed, and this has been noticed in many of our cases. While the above differential count is typical of the results obtained by some observers, we have found, even in a limited number of examinations, that it is not of sufficiently frequent occurrence to be depended upon in

reaching a diagnosis. In fact, in most of our counts we found that the small lymphocytes outnumbered the large in every stage of the disease.

Vedder, who made hundreds of blood counts upon patients suffering from the disease in the same epidemic as that from which we obtained our material, found that the polymorphonuclear leucocytes were greatly decreased, and the small lymphocytes greatly increased during every stage of the disease. He also found that the large lymphocytes are moderately increased during the latter days of the illness. His results will be published later in full.

Blood plates.—We have observed no changes in the number or appearance of the blood plates in dengue.

Blood plasma.—In neither fresh nor stained specimens of dengue blood have we been able to demonstrate any organism of etiological significance in the blood plasma. In a few instances bacteria were noticed, but from the ease with which blood cultures become contaminated in this climate, we believe that these bacteria were of external origin. The most common bacterium observed was a long, stout bacillus, actively motile, and commonly seen here in blood specimens from various sources. No organism resembling a protozoon was observed in the blood plasma. Yeast cells were frequent contaminations in stained specimens.

Summary.—From our examinations of the blood in dengue we consider that the following conclusions are justified:

1. There does not occur in the blood of dengue any visible organism, either bacterial or protozoal in nature, which can be considered as the cause of the disease.

2. Dengue is not accompanied by anemia, the red blood count being normal in uncomplicated cases. There are no characteristic morphological changes in the red corpuscles, leucocytes, blood plates, or blood plasma.

3. Dengue is characterized by a leucopenia, and, in the vast majority of instances, by a decrease in the polymorphonuclear leucocytes and a marked increase in the small lymphocytes; the increase in the small lymphocytes is constant throughout the disease.

BLOOD CULTURES.

With the exception of McLaughlin's researches which have been mentioned, and which, so far as culture methods are concerned,

were almost valueless, we have not been able to find in the literature any detailed descriptions of experiments having for their object the cultivation of bacteria or protozoa from the blood of dengue patients. In view of the success attained by Novy and others in the cultivation of trypanosomes, and by Rodgers, in cultivating the Leischman-Donovan body (*Herpetomonas donovani*), we were especially hopeful, that by applying similar methods in this disease, we might be able to secure growths of any protozoon which might be present. As we have stated, we were impressed with the idea that dengue might be caused by an organism belonging to the same group as those causing the relapsing fevers, or to some closely allied group, and we therefore endeavored, by employing special staining methods and culture media, to demonstrate such an organism.

Methods.—In our experiments we have used citrated blood and acid and alkaline broth as culture media. In making cultures with citrated blood, the sterilized syringe was first filled with citrate solution, which was then ejected, a very little being allowed to remain in the needle; the syringe was then filled with blood by plunging the needle into a prominent vein in the forearm of the patient and withdrawing the blood very slowly until the barrel of the syringe was full; the blood was then ejected into small sterilized glass tubes and kept at room temperature and in the lower compartment of the ice-box, the latter in order to give any organism undergoing a portion of its life cycle in a cold-blooded animal surroundings congenial to its development.

In making blood cultures in broth, 10 c.c. of blood, obtained from a vein of the forearm, were added to 250 c.c. of the bouillon, contained in 500 c.c. flasks, and incubated at temperatures of from 80° to 98° F.

Citrated blood cultures.—In eight cases we endeavored to secure cultures of the organism causing dengue by citrating blood from dengue patients, obtained at various periods of the disease. In none of the cases have we been able to demonstrate any organism in the culture which we can consider as of etiological significance; in none of the tubes of citrated blood did we observe any organism resembling, in the least, a protozoon, and all of the bacteria observed were evidently external contaminations; one, a small diplococcus, occurred in two of our cultures, but in the light of our later work with filtered blood, was evidently of no importance.

Broth cultures.—In twelve cases we used broth blood cultures, allowing them to incubate for as long as eight weeks. The majority of the flasks became contaminated, but in four case the blood cultures

did not show any growth at the end of eight weeks, when they were destroyed. A staphylococcus grew in one at the end of 48 hours, a diplococcus in three in 72 hours, accompanied by a large spore-bearing bacillus in two of the cases; a short, thick, motile bacillus together with a staphylococcus in one in four days, and various spore-bearing bacilli in the remainder. These organisms we regarded as contaminations and therefore did not experiment with them, the result of our work with filtered blood later confirming our opinion.

Summary.—As the result of our culture experiments we were forced to conclude that no organism was found constantly enough in our cultures to warrant us in regarding it as the cause of dengue, especially as a number of cultures remained sterile, though kept for as long as eight weeks. We observed no organism in any of our cultures which in the least resembled a protozoon.

INOCULATION OF DENGUE BLOOD.

Having thus failed to demonstrate any organism in either fresh or stained specimens of blood from dengue patients, or in our blood cultures, we directed our attention to the possibility of producing the disease in healthy men by the inoculation of blood from those suffering from dengue; fortunately for the success of our work, we were dealing with a disease which in the young and vigorous is not dangerous to life, and for this reason we felt justified in making the experiments. We hoped in this way to determine the presence or absence of the infective agent in the blood, for should such experiments prove successful they would demonstrate that the cause is in the blood, and that insect transmission, therefore is possible, whereas negative results would prove that the blood did not contain the organism unless it be one that first has to undergo a developmental cycle outside the body before it can infect man.

In order to secure subjects for experiment, a call for volunteers was issued to members of the Hospital Corps, U. S. A., serving at the Division Hospital, Manila, and as a result, four men volunteered, in all of whom we succeeded in producing dengue by intravenous inoculation of blood from cases of the disease. We desire to express our admiration of the courage and devotion to duty of these men, who, with no prospect of pecuniary reward, cheerfully placed themselves in our hands for these experiments.

As more men were needed, and as no more Hospital Corps men were available, we consulted Major-General Leonard Wood, commanding the Philippines Division, who authorized us to offer a reward for volunteering, as a result of which we secured more men than we could use, as we were limited to 16, including those already experimented upon. Unfortunately, of the 14 men* we have experimented upon to date, seven came from Fort William McKinley, having passed unharmed through the extensive epidemic of dengue at that post, and of these men we found two absolutely immune, three relatively immune, and one doubtful. Of the same number of Hospital Corps men who had not been exposed to dengue we found only one immune.

1. *Intravenous inoculation of unfiltered dengue blood.*—Eleven of our 14 volunteers were given intravenous inoculations of unfiltered dengue blood, and of these, seven developed the disease, while in one case the result was doubtful. In three of the cases there existed an absolute immunity to the disease, as proven by our experiments.

Experiment 1.

Case 1.—Chart 1. E. W. Private. Hospital Corps, U. S. A. Had not been exposed to dengue. At 3:30 P. M., July 24, 1906, he was given an intravenous injection of 20 minims of unfiltered dengue blood from Case 20 (see Chart A). The patient from whom the blood was taken for the inoculation had a mild attack of dengue and was probably nearly over the disease at this time. We believe that this accounts for the mild character of the experimental disease in this case, for while the symptoms present were typical, it will be seen upon referring to the temperature chart that the fever was slight as compared with our other experimental cases. The following is a résumé of the clinical record in this case.

July 24. At 3:30 P. M., inoculated intravenously with 20 minims of blood from Case 20. Subject in good health and temperature normal.
" 25, 26, and 27. Patient feeling well.
" 28. Has some fever and headache. Bowels constipated.
" 29. Patient feels uncomfortable, complaining of vague muscular pains and smarting of the eyes; eats and sleeps fairly well. Bowels regular.
" 30. Last night had severe headache, pain in eyes, in lumbar muscles, and in the elbows, ankles, and wrists. At present (11 A. M.) complains of dull headache and slight lumbar pain; the eyes are painful, the pain being aggravated by movement of the eyeballs. Tongue moist and clean.
" 31. Feels much better, the pain in the head and the muscles having disappeared. A slight eruption is present upon the back and chest.
August 2. Fells well. Eruption has disappeared.
" 6. Returned to duty.

*Of the fourteen soldiers who volunteered for this work, seven belonged to the Hospital Corps, U. S. A.; three to the Eighth U. S. Cavalry; two to the Sixteenth U. S. Infantry; one to the Thirteenth U. S. Infantry, and one to Company B, Engineer Corps, U. S. A.

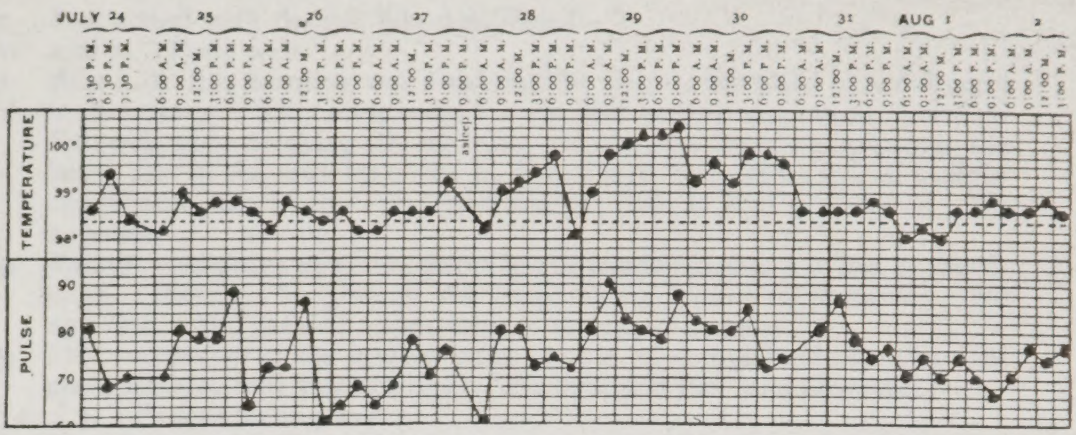


CHART 1.

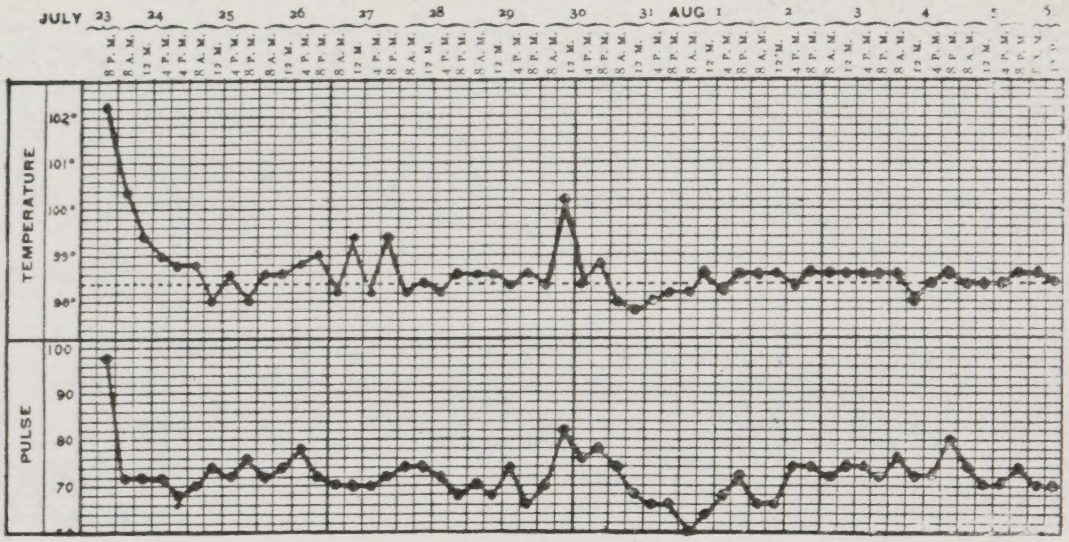


CHART A.

Remarks.—Upon reference to the temperature chart (Chart 1) it will be observed that the temperature began to ascend about 9 A. M. on July 28, but the patient complained of no symptoms until nearly 24 hours later. The incubation period in this case is, therefore, about 3 days and 18 hours, reckoning it from the morning of July 28. The eruption present in this case was a typical dengue eruption but was not very marked, only appearing over the chest and back.

Experiment 2.

Case 2.—Chart No. 2. W. R. H. Private, first class, Hospital Corps. Not previously exposed to dengue and in good health. At eleven o'clock, upon the morning of July 31, 1906, he was given an intravenous inoculation of 20 minims of blood from Case 30 (Chart B), who was suffering from a typical attack of dengue of four days' duration at the time the blood was taken. No symptoms appeared in Case 2 until early in the morning of August 3, as is shown by the following résumé of his clinical record:

July 31. Inoculated with dengue blood as stated.
August 1. and 2. Feels well.
" 3. At 2 A. M. this morning was awakened by pain in the chest and difficulty in breathing. This soon passed away and patient slept until morning. Felt well upon awaking this morning, but soon developed sharp pain in the head, and in the muscles of the back and legs. Complains also of a slight cough and pain in the eyes; had a slight chill at 5:30 P. M. today. No appetite.
" 4. Patient complains of severe pains in the lumbar region but has no headache. Diarrhea is present, the stool being watery in character. No appetite. His general appearance is typical of dengue.
" 5. Still complains of the lumbar pain; at 9 P. M. complained of abdominal pain, nausea, and vomited once. No eruption has been observed.
" 6 and 7. Patient feeling well.
" 8. Has severe headache and pain in the muscles and joints. Last night was very nervous, almost delirious.
" 9. Feeling well. Returned to duty August 14, 1906.

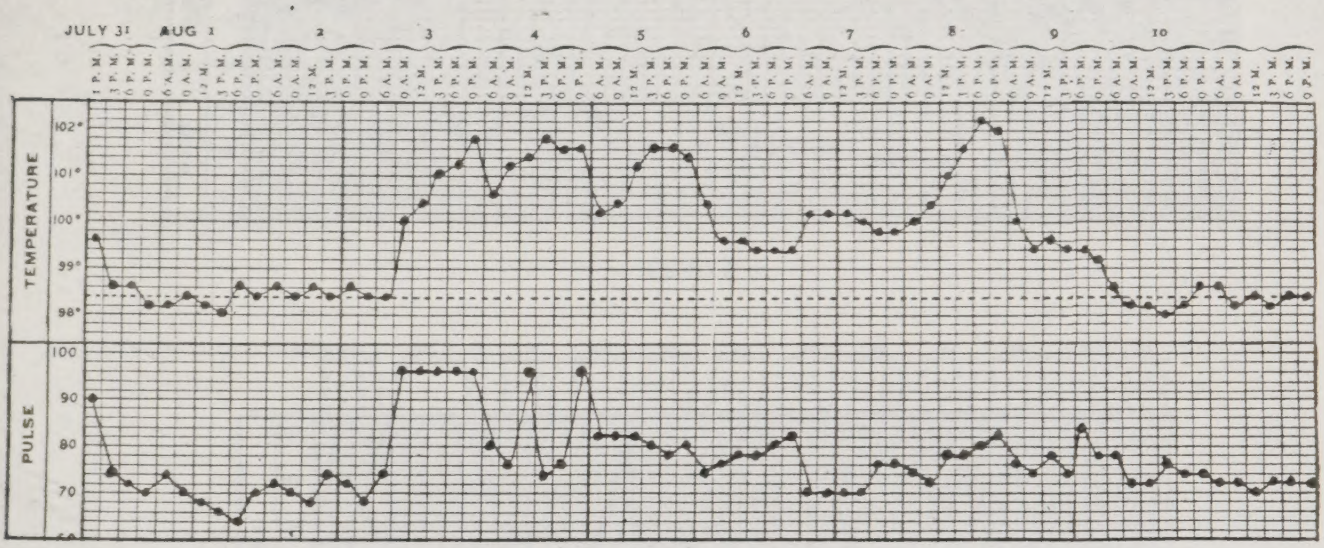


CHART 2.

Remarks.—In this case the incubation period, as shown by the temperature chart, was approximately 2 days and 19 hours. The temperature curve is typical of a moderately severe case of dengue fever and the symptoms corresponded; the terminal rise and fall is well shown in this chart. No eruption occurred at any time, although otherwise the symptoms were typical, with the exception of the diarrhea, which was present for a short time, accompanied by nausea and vomiting; these symptoms we regard as rather the exception than the rule in dengue, and we are inclined to believe that in this case they may have been excited by the presence in another tent of a case of cholera, a disease which just at this time was common in Manila.

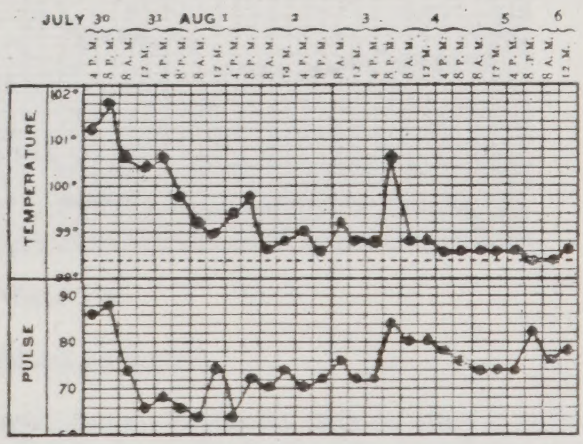


CHART B.

Experiment 3.

Case 3.—Chart 3. E. W. Private, first class, Hospital Corps, U. S. Army. At the time of inoculation this man was perfectly well and had not been exposed to dengue. He was given an intravenous inoculation of 20 minims of dengue blood at 2 P. M., August 16, 1906, from Case 36 (see Chart C). The latter case was a most typical one of dengue, which, at the time the blood was taken, had lasted a little over three days. The clinical record of Case 3 follows:

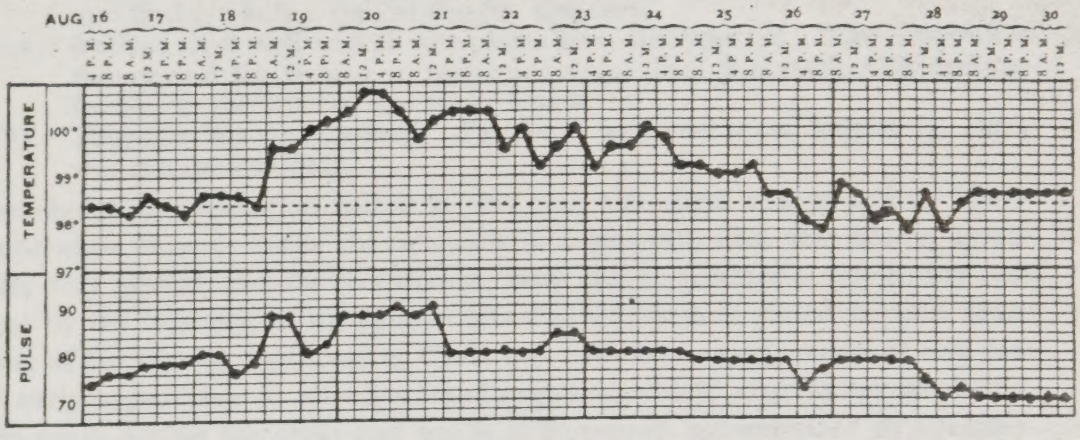


CHART 3.

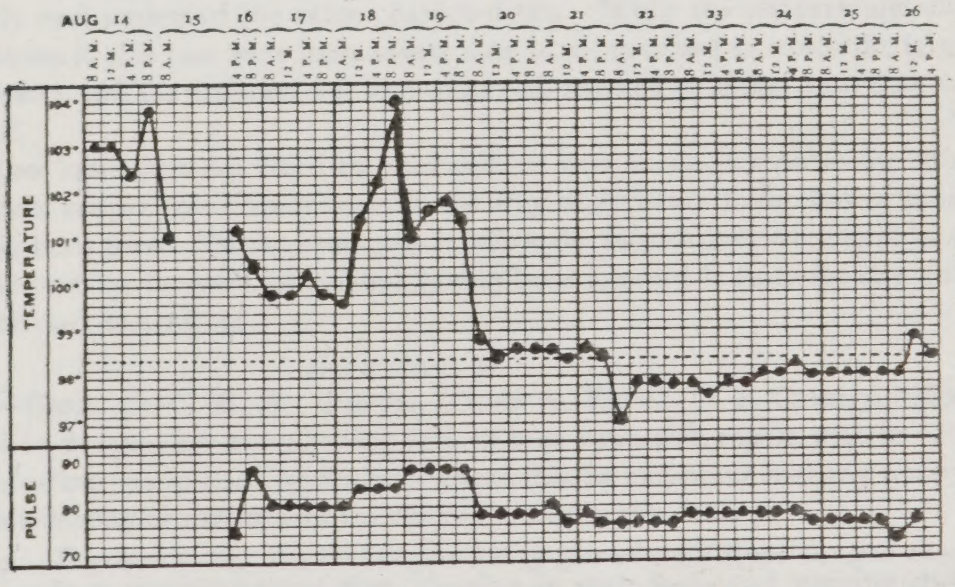


CHART C.

August 16. Inoculated with dengue blood as stated.
" 17. and 18. Feeling well.
" 19. Slight temperature last night, but no headache, pain or other symptoms.
" 20. Last night suffered from headache, pain in the bones and muscles of the back, and severe pain in the knee joints. This morning has headache located in the temporal region, and general muscular pain. Eyeballs pain and are tender to the touch. Stomach and bowels normal. An eruption appeared this morning, and covers almost the entire body but is especially marked over the trunk and arms; it consists of very fine, slightly elevated areas, dark red in color, the color disappearing upon pressure.

August 21. Feels better this morning, but still has pain in eyes, shoulders, knees, and wrists. The eruption is still present but is not as marked as yesterday. Appetite poor. Bowels regular. Tongue moist and clean.

" 22. Patient had more headache last night but feels much better this morning. The eruption is still present and itches greatly. The bowels are loose. Appetite good.

" 23. Had headache last night which caused insomnia, also aching pains, severe in character, in the muscles and joints. The eruption has greatly increased covering the entire body, being especially marked over the trunk and limbs. It is a typical dengue eruption.

" 25. Patient slept well and all symptoms have disappeared. The eruption is fading and the itching is very annoying; there is considerable desquamation in the form of fine white scales.

" 27. Eruption has disappeared. Patient is well and upon August 31, 1906, was returned to duty.

Remarks.—The incubation period in this case was about 2 days and 18 hours. The temperature curve is not as characteristic as is generally observed, but the symptoms were very typical, and the fact that we were able to produce a very severe case of dengue by the injection of the filtered blood of this patient proves beyond doubt the nature of the disease (see Case 9, Chart 9). An interesting feature of this case was the early appearance of a well-marked eruption, which, after fading gradually, increased again during the crisis and finally disappeared, accompanied by considerable desquamation. It is also interesting to note that in the case of dengue referred to as being produced by the injection of filtered blood from this case, the eruption appeared early and presented the same characteristics. While the temperature was low the symptoms in this case were more severe than in many others in which the fever was much greater, and the eruption was almost as well marked as in any case that we have observed.

In the three cases just described the inoculations were made as soon as the subject volunteered, no previous experiments in the way of exposure to fomites or mosquitoes having been tried. In the cases which follow the inoculations were used as a final test of immunity, the men inoculated having been exposed both to fomites and supposedly infected mosquitoes.

Experiment 4.

Case 4.—Chart 4. C. H. B. Private, Troop A, Eighth U. S. Cavalry. Had been exposed to dengue, his troop having had 18 men upon sick report with it. At time of volunteering was in good health, and stated that he had never had any serious illness. The following is a résumé of the clinical record in this case.

September 22. Exposed to fomites of dengue, being placed with three dengue cases in a mosquito-proof tent, sleeping in their beds and wearing their underclothes.

" 26. No results being obtained, the patient was placed under a mosquito bar with mosquitoes that had bitten Case 38, a typical case of dengue, the night before.

" 27 to Oct. 2. During this time the subject has slept under the mosquito-bar containing the infected mosquitoes, but claims that he has not been bitten at all and his statement is confirmed by the fact that the mosquitoes all remained empty during this time, and made no attempt to bite, most of them dying while in the mosquito bar. He states that so far as he knows he has never been bitten by a mosquito, although he has campaigned in localities in Africa which were almost uninhabitable because of these insects. At first we were inclined to doubt his statements in this respect but observation has convinced us that this man is really immune to mosquitoes.

October 3. At 10 A. M. the subject was given intravenously 20 minims of unfiltered blood from Case 44, a very typical case of dengue of about 3½ days duration.

" 4 and 5. Feeling well.

" 6. Last night, about 10:30 P. M., the patient complained of fever and muscular pains. This morning has severe pain in the head, back, and limbs. Face greatly flushed, conjunctivae congested. Bowels constipated. Tongue moist with thin white coating.

" 7. Vomited last night. Has less pain this morning, located mostly in the muscles of the loins and thighs. Has much headache and pain in the eyeballs upon moving them.

" 9. Feeling well.

" 10. Had headache and muscular pains last night. Feels better this morning.

" 15. Returned to duty.

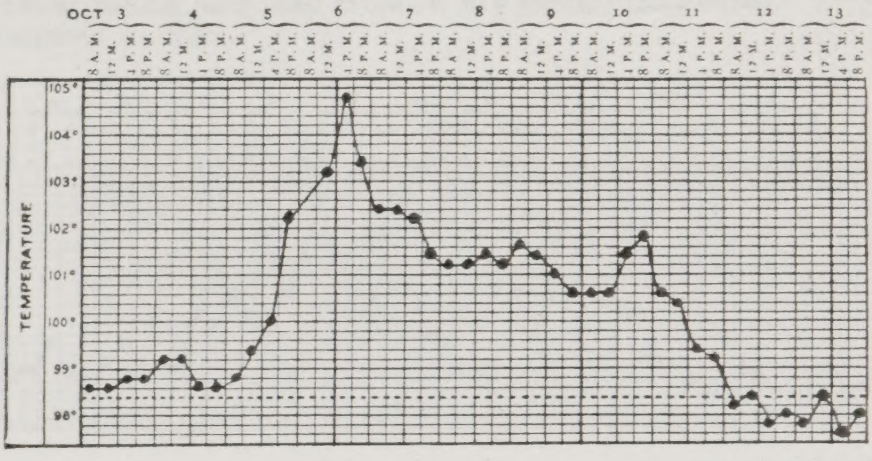


CHART 4.

Remarks.—The temperature curve in this case is one often observed in naturally acquired dengue, and the symptoms throughout were typical of the disease. The absence of an eruption is to be noted; not that it is unusual in many natural infections, but because in our experimental cases an eruption was almost always present. Thus of the 10 cases in which we were successful beyond doubt in producing dengue, eight presented well marked eruptions, while one was somewhat doubtful in this respect.

Experiment 5.

Case 5.—Chart 5. C. R. D. Second-class private, Co. B, Engineer Corps. U. S. Army. This man at the time of volunteering was in good health, but had been exposed to dengue during the Fort McKinley epidemic. He was observed for a period of four days before any experiments were made. The following is the clinical record of this case.

September 19. At 1 P. M. a small abrasion was made upon the mucous membrane of his cheek, and he then rinsed his mouth with diluted blood from a dengue case. No results were obtained from this experiment.

October 3. Has been exposed to fomites as described in Case 4, since September 22. No results obtained.

" 4. Patient slept last night under mosquito-bar containing mosquitoes that had bitten a typical case of dengue the night before. He was bitten several times during the next few nights but dengue did not develop.

" 22. At 3 P. M. today an intravenous injection of 20 minims of blood from a dengue case, Case 60, was given the subject.

October 23, 24, and 25. Feeling well.
" 26. At 7 P. M. today the patient complained of headache, pain in the lumbar region and in the legs, and loss of appetite.
" 27 and 28. Patient complained of severe headache, pain in the lumbar region and in the joints. His hands and wrists are swollen slightly, and his face, arms, and hands greatly flushed. He is constipated and has no appetite.
" 29. Feeling much better. An eruption has appeared upon the chest and abdomen, resembling more the eruption due to heat than a dengue eruption.
" 30 and 31. Patient feels well. There is a well-marked rash over the back and chest which upon the 31st had extended over the arms and legs. This rash is a typical dengue rash.
November 1. The rash is still well marked and is present over the entire body, including the palms of the hands and the soles of the feet.
" 2. Patient feeling well, and eruption has almost disappeared.
" 5. Returned to duty.

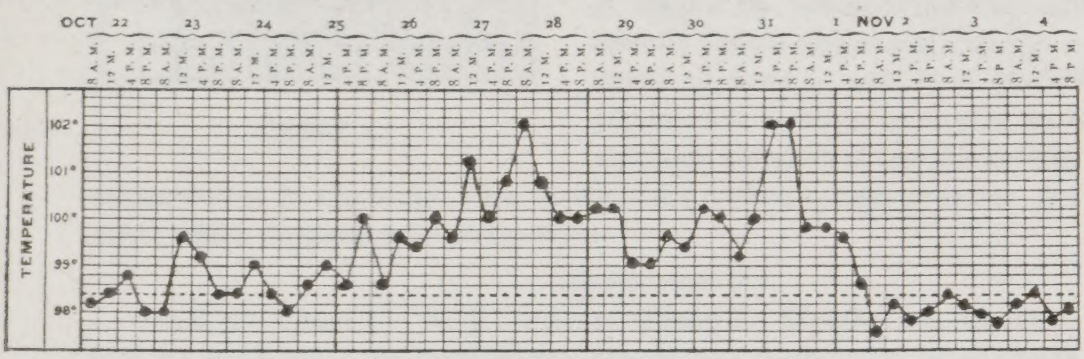


CHART 5.

Remarks.—It will be observed that this man did not contract dengue through the mucous membrane of the mouth, nor from mosquito bites. As regards the latter experiment, we cannot be sure that he was bitten more than one or two times by the mosquitoes, as they disappeared very rapidly from the bar in which they were confined. From the intravenous inoculation of unfiltered dengue blood he developed a very typical attack of the disease, as will be seen by referring to the temperature chart. The incubation period in this case was longer than in any of our previous cases, being four days and four hours.

Experiment 6.

Case 6.—Chart 6. J. E. S. Private, Troop H, Eighth Cavalry. At thetime of volunteering this man was in good health but had been exposed to dengue at Fort McKinley. The following is a record of the experiments performed in this case.

September 22. Exposed last night to mosquitoes that bit Case 41 on September 13. Says that none bit him during the night. September 23: exposed again last night to same mosquitoes and says he was bitten once. Dengue did not develop.
October 7. Was exposed to mosquitoes that bit Case 44 the night before.
" 8 to 15. Feeling well, and states that he does not know whether he has been bitten. Dengue did not result.
" 25. Exposed to mosquitoes that had bitten Case 60 the night before. Was bitten at least twice. Mosquitoes had disappeared from the bar by October 28, and he was not bitten again. Dengue did not result from this experiment.

October 31. Exposed to mosquitoes that had bitten Case 65 the night before. Dengue did not result.

November 8. At 3 P. M. today the patient was inoculated intravenously with 20 minims of dengue blood from Case 70. The man inoculated from had a typical attack of dengue fever, and the inoculation was made upon the third day of the disease. (Temperature 101.4°.)

" 10 to 14. Subject feeling well.

" 15. In the afternoon the subject had a slight chill accompanied by pain in the muscles and severe headache.

" Patient complains of severe headache, backache, and pain in the limbs.

" 17 to 20. During this time the patient presented the usual symptoms of dengue, which have already been indicated.

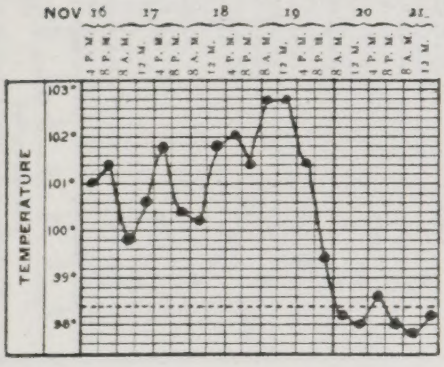


CHART 6.

Remarks.—The chief point of interest in this case is the long period of incubation, exceeding that of any experimental case that we have observed. Inoculation was made as 3 P. M., November 8, and the first symptoms appeared upon the afternoon of November 15, making the incubation period about seven days. It should be remembered that this man had already passed unharmed through a severe epidemic of dengue, and it is probable that he possessed a relative immunity to the disease, although his clinical symptoms were typical and rather severe in character. He developed a typical dengue eruption just before the crisis, which disappeared with the fever.

Experiment 7.

Case 7.—Chart 7. W. J. Private, Troop D, Eighth U. S. Cavalry. This case is of interest because the experimental dengue was complicated by an attack of malarial fever. The man had been exposed to dengue at Fort McKinley, but was in good health at the time of volunteering. The following is the clinical record of his case:

September 19 to 26. Exposed to fomites of dengue during this time in the manner described.

" 26. Exposed to mosquitoes that had bitten Case 80 (Chart L) the night before. This case was afterward found to be suffering from aestivo-autumnal malarial fever.

" 27 to 29. Subject feeling well.

October 8. Exposed to mosquitoes that had bitten Case 4 (Chart 4) the night before, and was bitten twice upon the night of October 9. Dengue did not result from this experiment.

" 25. Exposed last night to mosquitoes that had bitten Case 81 (typical dengue) the night before. States that he did not feel well during the night, and complains of headache.

" 26 to 28. Aestivo-autumnal parasites were found in his blood on the afternoon of October 27, and quinine was at once administered. Feels well upon the 28th.

November 17. Inoculated at 10:30 A. M., with 20 minims of blood from Case 82, who was suffering from a typical attack of dengue which had lasted about three days.

" 18 to November 23. Feeling well until November 31, when he says that he developed slight muscular pains which have persisted since.

" 24. At 10 A. M. patient had a slight chill followed by a high fever. Upon November 22 an eruption had been noticed covering the entire body, which resembled the eruption of dengue and which is still present.

November 26. Patient is feeling well and is free from pain. He states that he had had considerable pain before his chill for several days. He is covered with an abundant rash, which presents all the characteristics of that observed in our other dengue cases.

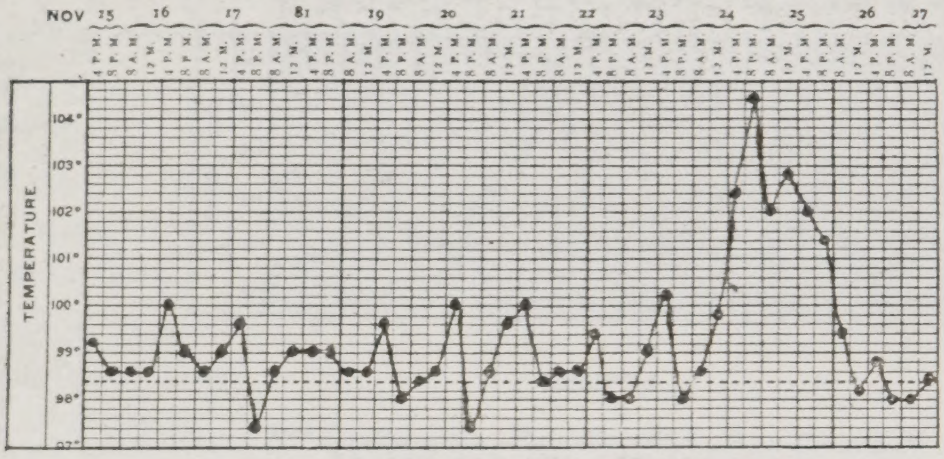


CHART 7.

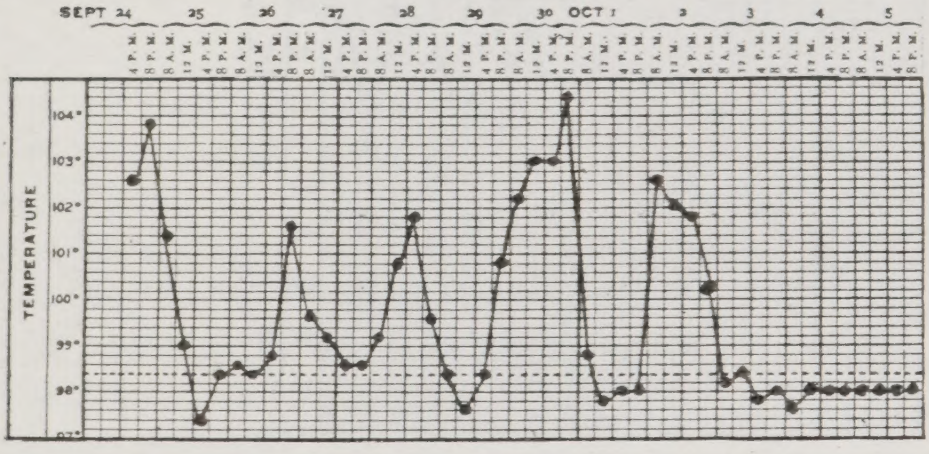


CHART L.

Remarks.—It is difficult in this case to determine the exact period of incubation, and the chart is also atypical because of the concurrent malarial infection. The clinical symptoms, however, were typical, and the presence of the rash removes all doubt as to the nature of the infection.

Experiment 8.

Case 8.—Chart 8. R. R. Private, Co. H, thirteenth U. S. Infantry. This man was in good health at the time of volunteering, but had been exposed to dengue during the epidemic at Fort McKinley. The following is the clinical record of this case:

September 12. Inoculated intravenously with one-half minim of blood from Case 83 (Chart O), who was suffering from a typical attack of dengue.

" 15. Patient states that he had a severe headache last night. Still complains of headache and pain in the arms and legs.

" 16. Still complains of headache, but has no other pain. Bowels constipated. Appetite poor.

" 17 to 19. Patient feeling well.

September 19. Inoculated intravenously at 1 P. M. with 1 c.c. of filtered blood from Case 2 (Chart 2). No result.

" 25. Inoculated intravenously at 1 P. M. with 20 minims of blood from Case 38. No result.

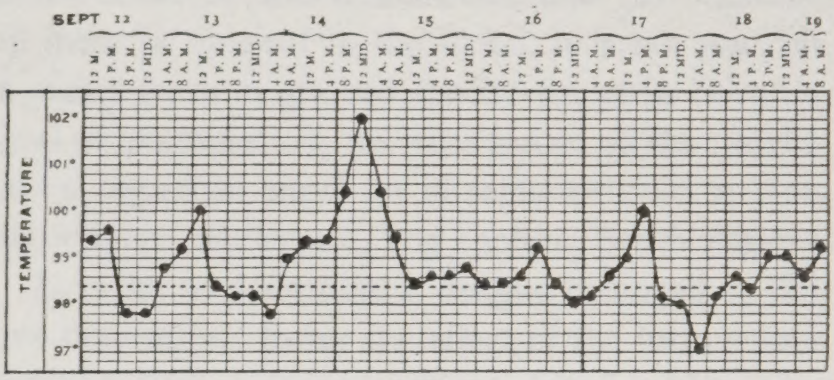


CHART 8.

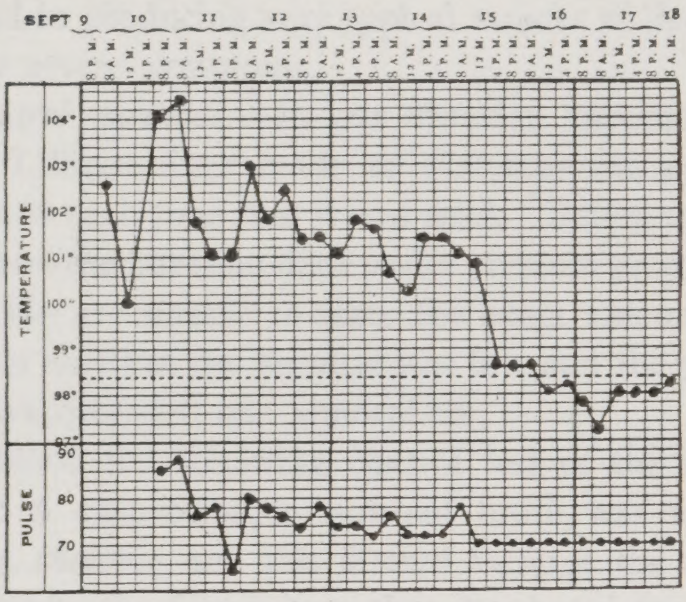


CHART O.

Remarks.—We have regarded this case as doubtful, although we are inclined to believe that the rise in temperature upon September 15 was due to a slight attack of dengue produced by the inoculation of the one-half minim of dengue blood. This is much less blood than we have used in our other experiments, and it may be that the slight symptoms produced may be due to this fact. If this man did not suffer from an attack of dengue as the result of his first inoculation, he must have been immune, as neither the subcutaneous injection of the filtered dengue blood or the intravenous injection of unfiltered dengue blood produced the disease.

Summary.—The intravenous injection of unfiltered dengue blood into healthy men is capable of producing a typical attack of dengue in such men. Thus of 11 men so inoculated, seven suffered from dengue fever, while in one the result was somewhat doubtful. Three of the men were absolutely immune to the disease.

2. *Intravenous inoculation of filtered dengue blood.*—Having proven by our inoculation experiments with unfiltered blood from dengue patients that the disease could be thus transmitted, and, furthermore, that while the cause must be present in the blood, it is not possible to demonstrate it in either fresh or stained specimens, or in cultures, by any known method of examination, we were forced to the conclusion that the causative organism must be ultramicroscopic in size, as in the case of yellow fever, rinderpest, hog cholera, South African horse sickness, etc. In order to determine if dengue belonged to this class of infections, we determined to try the effect of the intravenous inoculation of filtered blood from dengue patients into healthy men. We have experimented in this way upon two men, in both of whom we have been successful in producing very typical attacks of dengue accompanied by rather severe symptoms.

Filter used and control methods.—In our filtration experiments we have employed a Lilliput filter made of diatomaceous earth, which was carefully tested each time that it was used. Before using, the filter was sterilized and the filtration done under pressure produced by a water vacuum. We are unable to state the exact amount of pressure obtained in this way but the vacuum was low and the pressure used could not have exceeded one atmosphere.

After filtering the blood the following control test of the filter was made in each case: A suspension, in nutrient broth, was made of *M. melitensis* and *Sp. cholerae*, and this filtered through the filter used in filtering the blood; the filtrate was then incubated for two weeks, daily examinations of it being made during this time.

The filter we used retained both of these organisms, the filtrate remaining sterile for two weeks, when it was thrown away. Besides the control test of the filter, we kept, in each instance, a portion of the filtered dengue blood for a period of 10 days, making daily examinations, and in one case, we made several cultures from the filtered blood in broth, but no growth was obtained in either the filtered blood or the cultures.

Experiment 9.

Case 9.—Chart 9. E. J. D. Private, Hospital Corps, U. S. A. Upon August 21, 1906, 10 c.c. of blood were drawn from the median basilic vein of Case 2, an experimental case of dengue which has been described (see Case 2, Chart 2). The blood

was taken upon the third day of the disease, the symptoms of the patient at the time consisting of fever, headache, severe pain in the muscles of the shoulders and in the wrists and knees, while there was present a typical dengue eruption.

The blood was rapidly defibrinated, diluted with an equal amount of salt solution, and filtered through a Lilliput filter, controlled as described. The filtration of a sufficient quantity for use was completed in about three-quarters of an hour. Of the filtrate, 50 minims, containing 20 minims of the filtered blood, was inoculated intravenously into Case 9 at 4.20 P. M., August 21. The patient at the time of inoculation had been in the hospital under observation for several weeks, and as no cases of dengue had occurred in the hospital during that time, he had not been exposed to the disease. No symptoms of importance developed until August 25, the period of incubation being 3 days and 11 hours. Previous to the decided onset of the disease there had been slight fever and some pain in the back, symptoms which were probably due to a chronic gonorrhea from which he had suffered for some time. The following is the clinical record of this case:

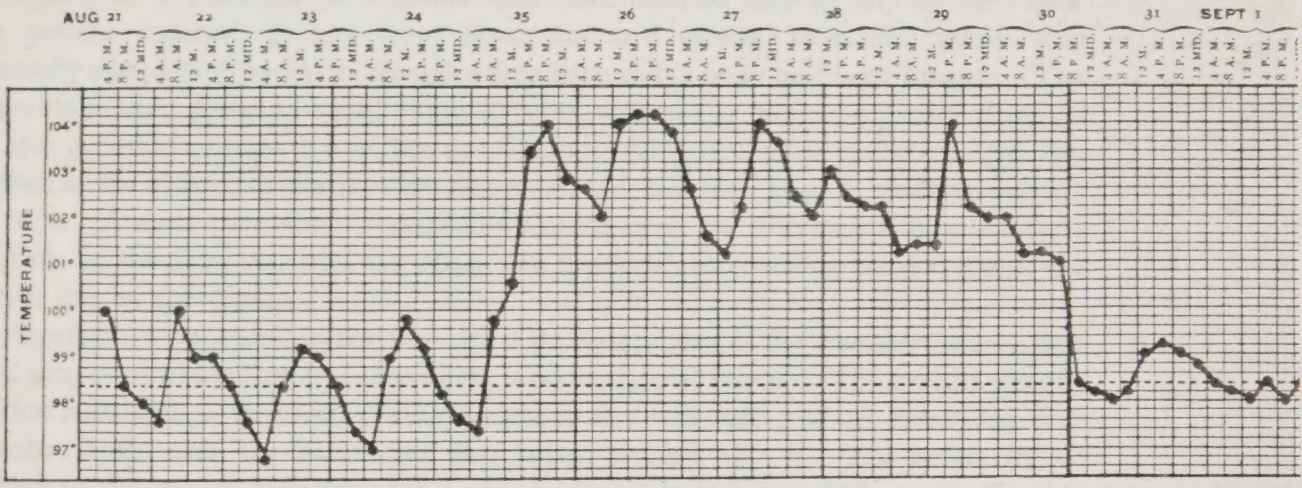


CHART 9.

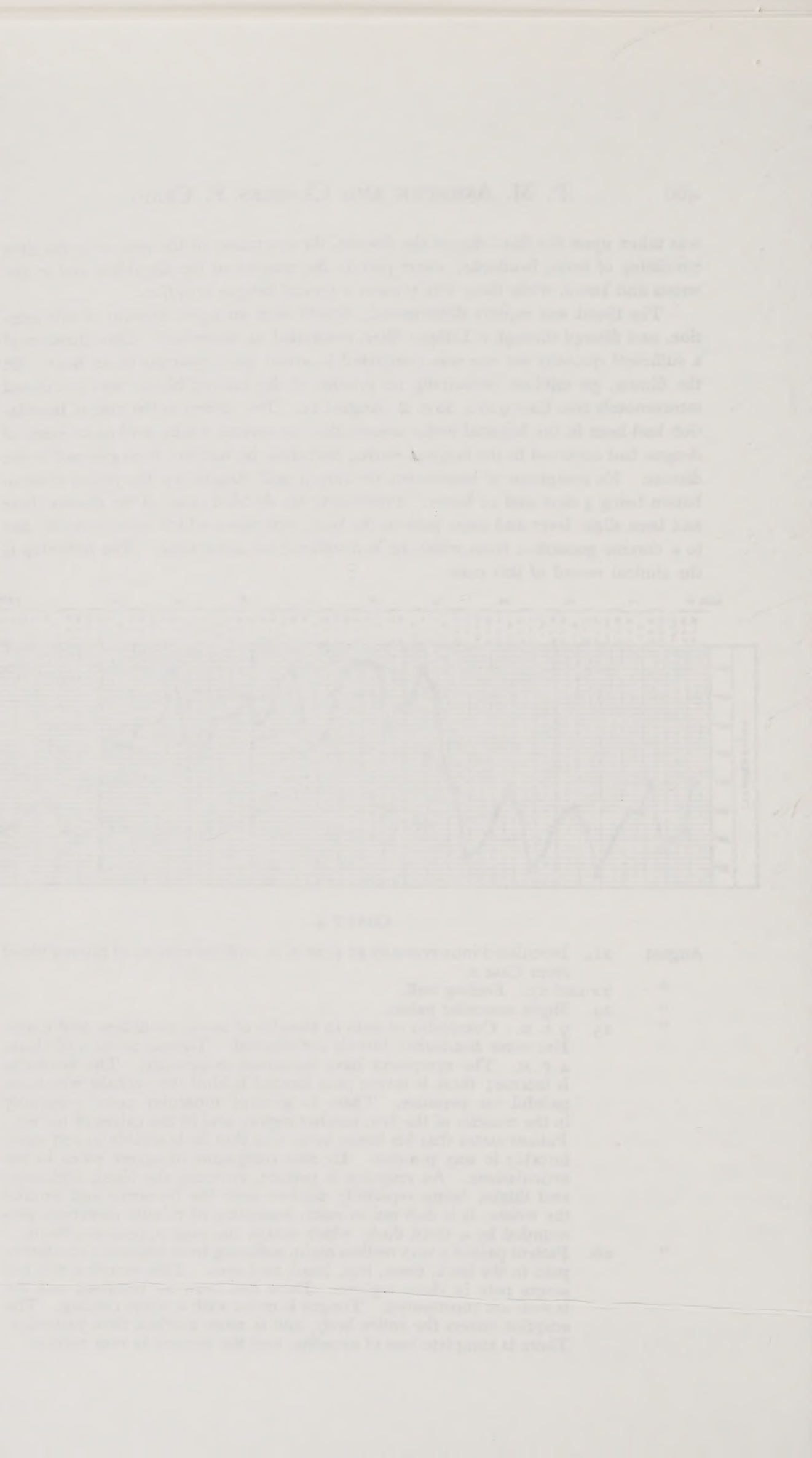
August 21. Inoculated intravenously at 4:20 P. M. with 20 minims of filtered blood from Case 2.

" 22 and 23. Feeling well.

" 24. Slight muscular pains.

" 25. 9 A. M. Complains of pain in muscles of neck, shoulders, and knees. Has some headache; bowels constipated. Tongue moist and clean. 4 P. M. The symptoms have increased in severity. The headache is intense; there is severe pain located behind the eyeballs which are painful on pressure. There is general muscular pain, especially in the muscles of the jaw, lumbar region, and in the calves of the legs. Patient states that his bones ache, and that he is unable to rest comfortably in any position. He also complains of severe pains in the articulations. An eruption is present, covering the chest, abdomen, and thighs, being especially marked over the forearms and around the wrists; it is dull red in color, consisting of minute elevations surrounded by a vivid flush, which makes the rash appear confluent.

" 26. Patient passed a very restless night, suffering from insomnia and severe pain in the back, chest, legs, head, and eyes. This morning still has severe pain in these regions. There has been no vomiting and the bowels are constipated. Tongue is moist with a white coating. The eruption covers the entire body, and is more marked than yesterday. There is complete loss of appetite, and the patient is very restless.



August 27. Feels much more comfortable this morning. The steady ache in the muscles has disappeared but he still suffers from lancinating pains in the head, back, and legs. The eruption has almost disappeared.
" 28. Passed another restless night, and suffered a great deal from pain in the muscles. This morning he complains of severe headache and pain in the loins and legs. The eruption has faded from the trunk.
" 29. Feels better this morning. Still has headache and pain in the eyes, but the general muscular pain has disappeared.
" 30. Still complains of pain in the head and eyes, but slept well last night.
" 31. Patient states that he feels very well this morning. Has no pain, and appetite is returning. There is present a very profuse dengue eruption covering the entire body, especially marked upon the arms, legs, hands, and feet.
September 1. Feels well. The eruption is less distinct, although it still covers the entire body.
" 2. Is feeling well in every way, and the eruption has disappeared.
" 4. Discharged from our experimental ward.

Remarks.—This case, as shown by the clinical record and the chart, is typical of severe dengue, but the initial eruption was more marked than in any of our cases. The patient suffered greatly from headache and general muscular pains, and repeatedly stated that he felt as though every bone in his body had been broken. The temperature chart presents a high range of fever, with not as marked a period of remission as is generally observed; it will be noted that morning remissions occurred regularly, but that in the afternoon the temperature ascended, reaching 104° F. on three successive days. A more permanent remission occurred upon the fifth day, succeeded upon the sixth by the final rise and the crisis, the temperature reaching normal upon the seventh day of the disease.

An eruption appeared in this case upon the second day of the disease (the so-called initial eruption) extending over the chest, abdomen, and thighs. The typical dengue eruption occurred, as is usual, during the crisis, and was very profuse, extending over the entire body, even the hands and feet being covered with it. The severe initial eruption in this case is very unusual, and it is most interesting to find, upon reference to the clinical history of Case 2, from whom this man was inoculated, that an eruption occurred in that case also upon the second day of the disease.

Experiment 10.

Case 10.—Chart 10. B. S. First-class private, Hospital Corps, U. S. Army. Upon August 31, 1906, at 12:15 P. M., this man, who had been on duty at the Division Hospital for weeks, had been carefully observed, and had not been exposed to dengue, was given an intravenous injection in the arm of $3\frac{3}{4}$ c.c. of normal salt solution containing 20 minims of filtered dengue blood from case No. 87 (Chart H). Ten cubic c.c. of blood was taken from the median basilic vein of Case 87, at 10:30 A. M., August 31, diluted with normal salt solution and filtered through the same filter used in Case 9, the filter being controlled as has been described. This filtered blood was used for the inoculation. The patient from whom the blood was obtained was suffering from a rather severe attack of dengue and the blood was taken upon the fourth day of the disease.

After inoculation with the filtered blood no symptoms appeared in Case 10 until midnight of September 3, but upon referring to the temperature chart it will be noticed that fever had been present for at least sixteen hours before it was complained of. If we assume that the first rise in temperature indicates the onset of dengue in this case,

the incubation period must have been about 2 days and 12 hours, while if the first symptom complained of by the patient be considered as marking the onset, the incubation period would be three days. We consider that the incubation period in this case was 2½ days. The following is a résumé of the clinical record of this case:

CHART 10.

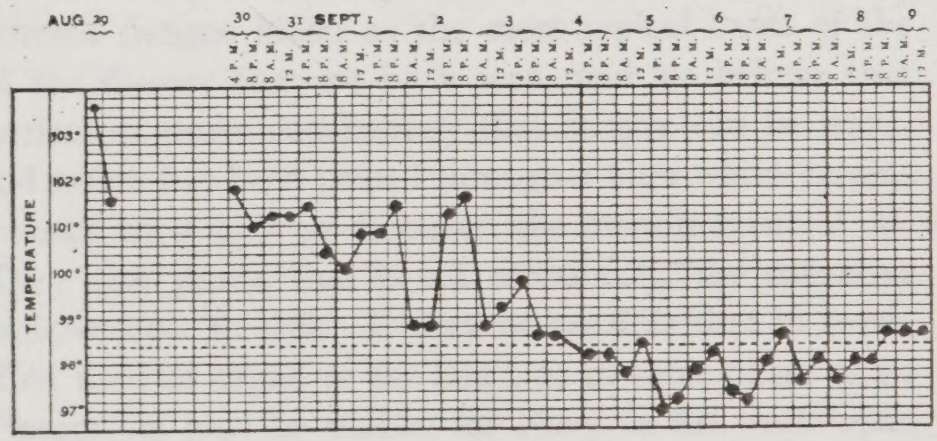


CHART H.

August 31. Inoculated as has been described.
September 1, 2, and 3. Feeling well.
" 4. Had a chill last night about midnight. This morning complains of pain in the muscles and bones, especially of the arms. His eyes ache and are much congested and the face is flushed. Has slight frontal headache and is very nervous. He complains of palpitation of the heart. Tongue moist and clean. Appetite poor.

September 5. Is feeling very nervous this morning, and was delirious last night. Has pain in head, back, arms, and legs. No appetite. Tongue moist, with heavy yellowish coating.

" 6. Spent a restless night, but is not so nervous this morning. Complains of severe pain in the back and legs. There is a faint, slightly elevated, sparse, macular eruption over the chest and back.

" 7. Patient had a comfortable night and this morning has but little pain. The eruption is well marked over the abdomen, chest, back, and arms, and is typical of dengue.

" 8. Feeling very comfortable. The eruption is fading a little.

" 9. Patient was delirious during the early morning hours, and is nervous and restless this morning, but free from pain. The eruption has largely disappeared.

" 10. Began to feel better at 4 P. M. yesterday, and now feels quite well. Slept well but perspired very freely during the night. The eruption has almost disappeared from the body, but is marked upon the forearms and wrists.

" 11. Feeling well. Eruption is fading slowly and there is a slight desquamation in patches.

" 15. Returned to duty.

Remarks.—The symptoms in this case were very severe, especially those connected with the nervous system. The subject of the experiment was of a highly nervous temperament, and this fact accounts, in our opinion, for the severity of the nervous symptoms.

The temperature curve in this case might be used as an illustration of the typical dengue curve, so perfectly does it agree with that described by every observer as characteristic of the disease. It should be noted, however, that the temperature is higher in this case than it usually is in naturally acquired infections or in our other experimental cases, with the exception of Case 9, also produced by the intravenous injection of filtered dengue blood.

We regard these two cases of dengue produced by the intravenous injection of filtered dengue blood as the most typical cases of the severe type of the disease that we have seen, and we believe that these two experiments prove conclusively that dengue can be transmitted by blood which has been passed through a filter which retains organisms as small as 0.05 μ in diameter, the measurement of *M. melitensis*. They also prove, that in all probability, the causative agent is ultramicroscopic in size. It may be possible that in some, as yet, unstudied fluid or organ of the body, or in some phase of its life cycle, the organism may be visible, for Novy, in his work upon *T. lewisi*, has proven that even so large a parasite as is this trypanosome exists in a form so small in cultures that it passes through a Berkefeld filter. While the same may prove to be true as regards the dengue organism, we feel justified in stating that, so far as present evidence goes, the parasite causing dengue fever is ultramicroscopic in size. This conclusion explains the uniformly negative results

obtained by nearly every trained observer in the search for the parasite of dengue. We conclude that a living organism is present in the filtrate, rather than a toxin, because of the length of the period intervening between inoculation and the appearance of symptoms (the incubation period), and also because we have reproduced the disease by the inoculation of the blood of experimental cases.

There is one point deserving of especial consideration in the discussion of these cases of experimental dengue by the injection of filtered blood, and that is, the relatively greater severity of the symptoms present. In both of these cases the symptoms were more intense in almost every particular than in any other of our experimental cases, despite the fact that no greater amount of blood was inoculated in these cases. This fact is very difficult of explanation, and we must confess to our ignorance of the cause. It may be that the admixture with salt solution or the time consumed in filtration, or both, acts in some way to increase the virulence of the organism, or that conditions favorable to its extra-corporeal development are present during the process of filtration which result in a more virulent form of the organism, though we have no evidence to offer in this respect.

Experimental transmission of dengue by the mosquito.—We have already mentioned the experiments of Graham regarding the transmission of dengue by the mosquito, in which he seemed to have proven conclusively that such a method of transmission is possible; we have also noted the negative results obtained by Carpenter and Sutton, Guiteras and Cartaya, and Agramonte, all of whom believe, however, that the mosquito is the active agent in the spread of the disease. To one who studies carefully the epidemiology of dengue, the conclusion is almost inevitable that this disease, which so closely resembles yellow fever and malaria in this respect, must also be transmitted by some species of mosquito. Its seasonal prevalence; its occurrence along low-lying, moist coast regions, and in the valleys of rivers, most frequently; its rapid diffusion in some localities, and its lack of diffusion in others; its relation to changes in temperature and moisture; its manner of spread from building to building in infected places; its absence in high altitudes where mosquitoes are absent; the presence of multitudes of mosquitoes wherever dengue occurs, and the absence of the disease in regions where mosquitoes are absent or few in number,

and the cessation of the epidemic in badly infected districts when conditions arise which are unfavorable to the propagation of mosquitoes, all point to some species of this insect as the infecting agent.

Accordingly, having demonstrated by the intravenous injection of dengue blood that the cause of the disease is present in that fluid, and that the parasite is probably ultramicroscopic in size, we turned our attention to the subject of mosquito transmission. Unfortunately for the fullest success of our work in this direction, we were forced, because of lack of other volunteers, to use a number of men who had already passed unharmed through the epidemic at Fort McKinley, and the majority of whom were immune, as proven by the negative result of inoculation of dengue blood. Thus, of the nine men in whom we endeavored to produce dengue by exposing them to the bites of infected mosquitoes, three were proven in this way to be absolutely immune, one may have had a slight attack of dengue previous to exposure, while three probably possessed a relative immunity, for while they developed dengue from the injection of a comparatively large amount of dengue blood, the symptoms were mild in character, and in one case the incubation period was greatly prolonged. In one case already described (see Case 4), no immunity to the disease existed, but the mosquitoes refused to bite the man.

The mosquito used.—In looking over the geographical distribution of dengue and of various species of mosquitoes, we found but one species of this insect that apparently occurred wherever dengue did, i. e., *Culex fatigans*. We do not wish to be understood as stating conclusively that this mosquito is the only one which may be present in dengue-infected localities, but only that, so far as we have been able to determine from the literature available, this species is constantly found and is mentioned by almost every recent investigator as being very numerous during epidemics of this disease. In Theobald's monograph the map illustrating the distribution of *Culex fatigans* might almost be used to illustrate the distribution of dengue fever, and if to this map be added the regions in which this mosquito has been demonstrated since it was published, the association of dengue and *Culex fatigans* is still more striking

For this reason and because this mosquito was used by Graham in his experiments, we decided to work with this species at first,

and in the event that our results were negative, to extend our work to embrace other species.

We have used mosquitoes reared in captivity, and also those caught in natural surroundings. In our successful case produced by the mosquito, however, we used insects reared by us from the egg, and thus we are sure that no infection occurred in these insects before they bit the dengue patient.

Our mosquito experiments were conducted as follows: The patient suffering from dengue was placed in bed beneath a mosquito-bar in a mosquito-proof tent. At night from 30 to 50 mosquitoes were liberated beneath the mosquito-bar and collected in the morning; almost invariably all of the mosquitoes left alive had bitten and were full of blood. The subject to be experimented upon, having been placed in bed, beneath a mosquito-bar in a mosquito-proof tent, the mosquitoes that had bitten the dengue case the night before were liberated beneath the bar, and orders given that the man remain beneath the bar until the mosquitoes had all disappeared; later we allowed the men to remain out of bed during the day, the mosquitoes being confined beneath the spread bar. With one exception, which has been noted, all of the men were bitten a few times, but in most instances the mosquitoes died or were killed before the men were bitten severely. We also confined mosquitoes that had bitten dengue cases in glass jars, and kept them for from four to six days before allowing them to bite, but in the few instances in which we tried this method our results were all negative.

We do not consider it necessary to give in detail our negative experiments, as they are all referred to elsewhere in this report, and we will only describe here the case in which we were able to produce dengue by the bites of infected mosquitoes.

Experiment 11.

Case 11.—Chart 11. B. L. W. Private, Hospital Corps, U. S. Army. This man had been on duty at the Division Hospital for several weeks, and as no cases of dengue had occurred in the hospital, had not been exposed to the disease. Upon September 12, 1906, the man being in good health, he was placed in bed under a mosquito-bar containing mosquitoes that had bitten Case 88 (Chart R) on the night of September 11, 1906. Case 88 was suffering at the time from a typical attack of dengue and the mosquitoes bit upon the third day of the disease. Case 11 was not bitten by the mosquitoes until the night of September 13, so far as he knows, and developed no symptoms until the night of the 17th, but upon reference to his chart it will be seen that he

had fever for nearly 24 hours before he noticed any symptoms. If we assume the period of incubation to be the period intervening between the 13th, the night upon which he was first bitten, and the 16th, when he had his first rise in temperature, the incubation period would be about $3\frac{1}{2}$ days. If, however, we assume the disease to have commenced when he first noticed symptoms, the incubation period would have been a little over four days. The following is a résumé of the clinical history in this case:

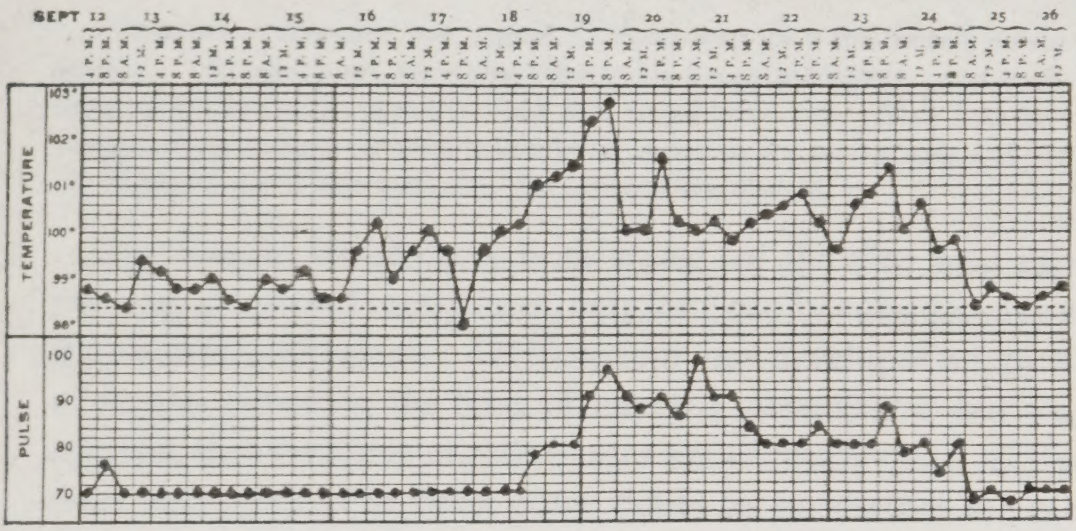


CHART 11.

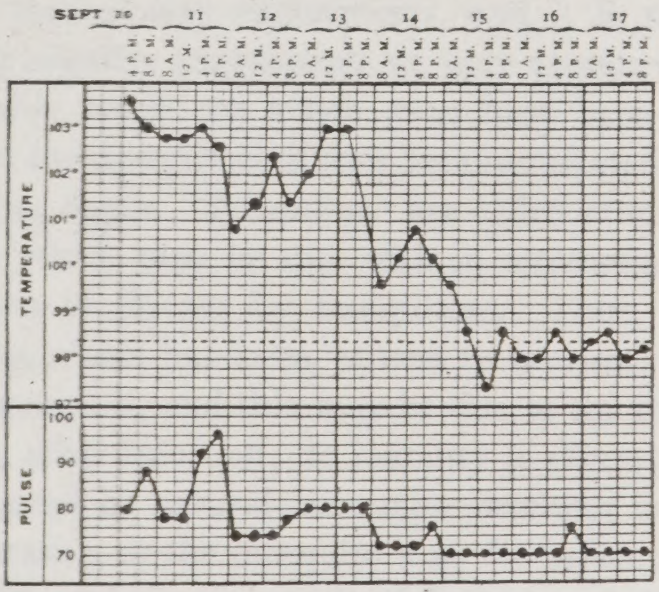


CHART R.

September 12. Put under net with mosquitoes that bit Case 88 last night.
" 14. Bitten by mosquitoes night of the 13th.
" 18. Had headache and felt uncomfortable last evening. This morning complains of headache and dull pain in the muscles and articulations.
" 19. Still complains of headache and general muscular pain and soreness. His face and eyes are greatly congested. Tongue moist and coated with thin white coat in center. Appetite poor. Constipated.

September 20. Last night had severe pain in the head, eyes, muscles of the back, but feels much better this morning.
" 21. Is feeling better. A faint rash is visible, covering the chest and abdomen.
" 22. Complains of soreness and stiffness in the muscles. The eruption is now plainly visible and is a typical dengue eruption.
" 24. Patient feels well. The eruption has almost disappeared.
October 1. Returned to duty.

Remarks.—This case was typical, in every way, of a moderately severe attack of dengue. The symptoms were those observed in the great majority of naturally acquired infections, and the temporature chart is a very typical one. This man had not been exposed in our dengue hospital before being bitten by the mosquitoes, and did not leave the mosquito-proof tent until after the onset of the disease.

For reasons which have been stated, of the nine men exposed to the bites of infected mosquitoes, only four can be considered in estimating the results obtained. Of these four, one developed a typical attack of dengue following the bites of infected mosquitoes; but we do not consider that the three negative cases are of much value, as the conditions were such as to cause some doubt as to whether the men were bitten by the mosquitoes.

It is obvious that many factors have to be considered in making mosquito transmission experiments, and it is more than probable that in our negative experiments we were unsuccessful in reproducing the favorable conditions which must have been present in Experiment 11, or the mosquitoes, if they became infected, may have perished before biting again. Schaudinn[9] has called attention to some of the difficulties which may be met with in attempting the experimental transmission of disease by mosquitoes. Thus, certain individuals of a species which has been proven to transmit the disease are not able to transmit it, and this may be due to the insect itself suffering from some other infection; to inability to digest the ingested blood; to an acquired or natural immunity resulting in the death of the specific parasite, or the mosquito may die before it has bitten again.

It is evident from the result of Experiment 11, that the parasite causing dengue does not undergo any cycle of development within the mosquito, unless it be a very short one. We are, therefore, of the belief that the parasite of dengue is one capable of living in the stomach of the mosquito for an unknown period of time, where it retains its virulence; that infection may occur at any time after the insect has ingested blood containing the parasite, so long as it remains virulent,

and that it is introduced into man when the insect bites, being regurgitated through the esophagus and proboscis with the fluid from the stomach. This theory is borne out by the results recently obtained by the Indian Plague Commission[10] in its remarkable study of the transmission of plague from rat to rat by the flea *Pulex cheopis*, and by the excessive rapidity of the diffusion of dengue, which would be impossible were the parasite one which underwent a prolonged developmental cycle within the mosquito.

We have dissected and examined a large number of mosquitoes that had bitten dengue cases, but we have never found any organism in the stomach or tissues suggestive of a stage in the life cycle of a protozoon. We cannot confirm Graham's results in this respect, and we believe that in the mosquito, as well as in the blood of infected man, the dengue parasite is ultramicroscopic in size.

By reason of lack of suitable volunteers and the subsidence of the epidemic, we have been forced to bring our mosquito experiments to a conclusion for the present. We have been unable to investigate many interesting questions regarding the transmission of dengue by the mosquito, such as the length of time the insect remains infective, the most infective period of the disease as regards transmission in this way, and whether transmission is purely mechanical or depends upon the development or multiplication of the parasite within the mosquito. All of these questions are of great importance to a correct conception of the etiology of dengue, and there would appear to be no good reason why, in regions where the disease is common, they should not be thoroughly investigated. We realize that the work that we have been able to do as regards mosquito transmission is very incomplete and that a very great deal remains to be done before this feature of the etiology of dengue is fully elucidated, but we believe that we have confirmed Graham's results in this respect and that we have proven experimentally that this disease can be transmitted by the mosquito, *Culex fatigans*. We also believe that mosquito transmission is the only natural method which has been proven by experiment, and that all of the epidemiological data confirm such a method of transmission.

EXPERIMENTAL PERIOD OF INCUBATION.

As will be seen from a study of the epidemiology of dengue the incubation period has been stated as varying from 24 hours to 10 days,

most observers regarding it to be from three to five days. The following table gives the period of incubation in nine of our experimental cases of the disease:

No. of Case	How Produced	Incubation Period
Case 1	Inoculation of unfiltered blood	3 days 18 hours
" 2	" " " "	2 " 19 "
" 3	" " " "	2 " 18 "
" 4	" " " "	2 " 12 "
" 5	" " " "	4 " 4 "
" 6	" " " "	7 "
" 9	" " filtered "	3 " 11 "
" 10	" " " "	2 " 12 "
" 11	By mosquito. About,	3 " 16 "

From the above table it will be seen that the incubation period of dengue in experimental cases of the disease varied from $2\frac{1}{2}$ days to 7 days, the average being about 3 days and 14 hours. This is practically what is stated by most clinical observers as the incubation period most frequently seen in naturally acquired infections. We have observed no case in which the incubation period was as short as 24 hours, and from our experiments we very much doubt the occurrence of such a short period of incubation.

IMMUNITY AND SUSCEPTIBILITY.

There is considerable confusion existing in regard to these points, the general trend of opinion being that almost everybody is susceptible to dengue, and that an attack produces immunity for a short time only. As to the latter point, i. e., the duration of acquired immunity, we cannot express a very positive opinion, as we endeavored, except in one case, to avoid the use in our experiments of men who had previously had dengue. In the one case noted as an exception dengue was induced though the patient said he had had three attacks, the last, two and one-half years before. We have also known a few other cases in which the disease developed naturally after a like period of time. The correctness of reports of cases in which attacks have been said to occur a month apart we very much doubt. We had about six patients sent back to us after such periods, supposed to be suffering from second attacks, but in no case was it so. The "second attack" was usually a malarial paroxysm.

As to natural immunity, we know that it occurs, or at any rate, that it may be temporarily present. We think it altogether probable that in many cases it may be relative, i. e., a small dose of the virus

may not be sufficient to overcome it, but a large one may. In one of our cases (Case 8) we were unable to decide positively whether an immunity which was present at the time of his discharge was natural or was acquired from a very light attack of the disease following inoculation with a half-minim of blood, though we incline to the latter belief. In at least one instance, immunity was apparent and not real; that is, the patient did not develop dengue when exposed to infected mosquitoes, but it was really due to the fact that the man was immune to mosquito bites. Later he developed dengue from the intravenous injection of dengue blood, and during the time he was sick the mosquitoes bit him as frequently as others about him; fortunately, his immunity to mosquito bites, so valuable in the tropics, was not permanently lost, for the patient now states that he is as free from mosquito bites as before he had dengue.

Our knowledge as to natural immunity cost us rather dear, as we were paying all our subjects of experiment, and did not relish exhausting the funds at our disposal in payments to men not capable of developing the disease. In the light of subsequent events we think that we made a mistake in accepting volunteers from Fort McKinley, where an epidemic of dengue had been and was prevailing, because, while we did not begin experiments upon the men until they had been under our observation and free from exposure for periods varying from a week to three weeks, and thus avoided the error of thinking the disease due to our inoculations when it was in reality due to other causes, we picked men some of whom had probably escaped natural infection because of their natural immunity.

As we have stated, three of our subjects were absolutely immune to dengue. Our assumption that failure to develop the disease after inoculation with 20 minims of blood from a dengue case constitutes absolute immunity is arbitrary, but seems justified by the constancy and severity of the symptoms produced in the successful cases.

Three of the men possibly showed a relative immunity, i. e., the amount of virus transferred to them by mosquitoes was not sufficient to cause the disease, though the intravenous injection of 20 minims of dengue blood was sufficient to do so. Possibly this relative immunity was only apparent, because we know that these men were not severely bitten by mosquitoes, and we do not know that the particular mosqui-

toes that did bite them might not have been laboring under some disability that prevented their transmitting the disease. It is noteworthy that two of these cases suffered from very mild experimental attacks of the disease, and that the third, while an ordinary case, presented an incubation period longer than the average.

Six cases, and if we count the doubtful case already described, seven, presented no immunity; that is, they developed dengue following the first attempt at inoculation. One case, immune to mosquito bites, showed apparent immunity, but developed dengue after the first inoculation.

Natural immunity and the practice of sleeping under mosquito-bars effectually protect a large proportion of healthy men from infection. Thus, in the Fort McKinley epidemic, the highest percentage of infections occurring in any one company was 58 per cent. The next highest was 52 per cent, and in the other companies it was lower. It must be remembered that in this epidemic no special measures were taken to prevent the spread of the disease, and the mosquito protection afforded consisted merely of the ordinary routine use of bars during the sleeping hours.

IMMUNITY AS SHOWN BY EXPERIMENT.

The following cases whose clinical records are here given were proven by experiment to be absolutely immune to dengue. The temperature charts are not reproduced as they contain no data of interest.

Case 12.—W. H. O. First-class private. Hospital Corps. U. S. Army. This man was on duty at the Division Hospital at the time of the experiments, and had never had dengue.

Experiment 1. On the night of September 12 the subject slept under a mosquito-bar with mosquitoes that had bitten a dengue case the night before. No symptoms of dengue developed.

Experiment 2. On the night of September 28 the subject was again exposed to mosquitoes that had bitten a dengue patient the night before. He was bitten repeatedly during the next few nights, but no symptoms of dengue developed.

Experiment 3. On October 3, the subject was inoculated intravenously with 20 minims of unfiltered dengue blood from Case 44. No symptoms of dengue developed, and the man was returned to duty October 11, 1906.

Case 13.—J. G. Private, Co. I, Sixteenth U. S. Infantry. This soldier belonged to a company of the Sixteenth Infantry that had sent twelve cases to hospital with dengue before this man volunteered. He had, therefore, been exposed to the disease.

Experiment 1. On September 12, 1906, the subject rinsed his mouth with normal salt solution containing 12 minims of dengue blood. The result of the experiment was negative.

Experiment 2. On September 19, the subject was given an intravenous inoculation of 20 minims of filtered blood from Case 11 (Chart 11). No symptoms developed.

Experiment 3. On the night of October 4, the subject was exposed to mosquitoes that had bitten Case 44 the night before. Dengue did not develop.

Experiment 4. On the night of October 15, the subject was bitten many times by mosquitoes that had bitten a dengue case two nights before. The result was negative.

Experiment 5. On October 22, the subject was given an intravenous injection of 20 minims of unfiltered blood from Case 95 (typical). No symptoms of dengue developed, and the man was returned to duty October 29, 1906.

Case 14.—J. B. P. Private, Co. M., Sixteenth U. S. Infantry. At the time he volunteered the company to which this man belonged had sent ten men to the hospital with dengue.

Experiment 1. On the night of September 24, 1906, the subject was exposed to mosquitoes that had bitten Case 11 (Chart 11) the night before. The result of the experiment was negative.

Experiment 2. The subject was exposed October 26 and 27 to mosquitoes that had bitten a typical case of dengue on October 25. The result of the experiment was negative.

Experiment 3. On November 17 the subject was given an intravenous injection of unfiltered blood from Case 82, who was suffering from a typical attack of dengue. No result attended this experiment and the man was returned to duty November 23, 1906.

Remarks.—These men were all exposed to fomites in addition to the experiments outlined, and we believe that the result of these experiments demonstrates that absolute immunity to dengue is present in certain individuals.

CONTAGION IN DENGUE FEVER.

We have carefully studied this portion of our subject because of its great practical importance, and believe that the following facts conclusively prove that dengue is not contagious in the least degree.

1. At the hospital at Fort William McKinley over six hundred cases of dengue were treated in the general wards without a single case originating among the other patients in the same wards. Only four men belonging to the Hospital Corps on duty at this hospital contracted the disease, three of them being night nurses on duty in the wards and the other a cook having no contact with the dengue patients. No precautions were used to prevent contagion other than the rigid use of mosquito-bars at night, the dengue and other patients eating together, and being closely associated throughout the day. It is noteworthy that the only men unprotected by mosquito-

bars at night, i. e., the three night nurses, all developed the disease.

2. In our dengue hospital, where we treated over 120 cases of the disease, no instance of infection occurred among the attendants, although their association with the dengue patients was very intimate and continued for over four months.

3. Our experiments with fomites were all negative. We endeavored to produce the disease by the exposure of healthy men to fomites, the men experimented with living in mosquito-proof tents with patients suffering from dengue, throughout the entire course of the disease. These men slept in the dengue patients' beds, wore their underclothes and pajamas, and ate and drank from the same table furniture, but in no instance did any of the men develop dengue. In this way we experimented with eight men, in some instances the exposure lasting for as long as two to three weeks.

We conclude, therefore, that dengue is not a contagious disease, and that patients suffering from it may be placed in the general wards of a hospital without fear of spreading the infection, provided precautions are taken to protect the patients from mosquitoes.

CONCLUSIONS REGARDING THE ETIOLOGY OF DENGUE.

From our study of the etiology of dengue, we believe that the following conclusions are justified:

1. No organism, either bacterium or protozoon, can be demonstrated in either fresh or stained specimens of blood with the microscope.

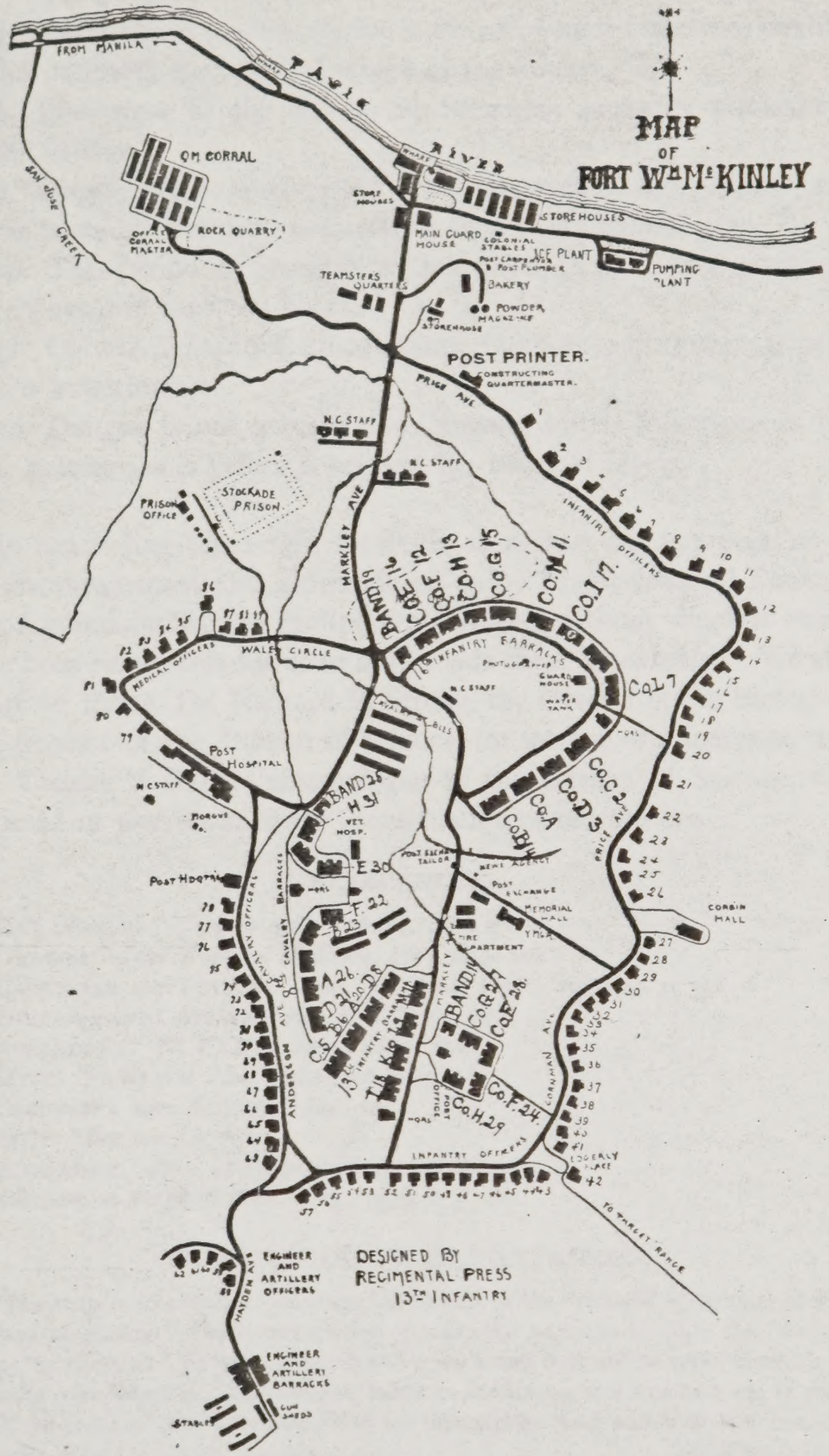
2. The red-blood count in dengue is normal.

3. There occur no characteristic morphological changes in the red or white corpuscles in this disease.

4. Dengue is characterized by a well-marked leucopenia, the polymorphonuclear leucocytes being decreased, as a rule, while there is a marked increase in the small lymphocytes.

5. No organism of etiological significance occurred in broth or citrated blood cultures.

6. The intravenous inoculation of unfiltered dengue blood into healthy men is followed by a typical attack of dengue.


MAP
OF
FORT WmMcKINLEY
FROM MANILA
PASIG RIVER
QM CORRAL
ROCK QUARRY
SAN JUAN CREEK
STORE HOUSES
MAIN GUARD HOUSE
COLONIAL STABLES
ICE PLANT
PUMPING PLANT
TEAMSTERS QUARTERS
BAKERY
POWDER MAGAZINE
POST PRINTER.
CONSTRUCTING QUARTERMASTER.
PRICE AVE
N.C. STAFF
STOCKADE PRISON
PRISON OFFICE
MARKLEY AVE
INFANTRY OFFICERS
BAND 19
Co E 12
Co F 13
Co H 15
Co G 15
Co M 11
Co I 17
13th INFANTRY BARRACKS
GUARD HOUSE
WATER TANK
Co L 17
MEDICAL OFFICERS
WALES CIRCLE
POST HOSPITAL
MORGUE
BAND 28
H 31
VET. HOSP.
Co C 2
Co D 3
Co A
Co B 4
E 30
F 22
POST EXCHANGE TAILOR
NEWS AGENCY
POST EXCHANGE
MEMORIAL HALL
YMCA
FIRE DEPARTMENT
CORBIN HALL
POST HDQTRS
CAVALRY OFFICERS
CAVALRY BARRACKS
ANDERSON AVE
A 26
C 25
D 21
B 6
A 20
D 8
13th INFANTRY BARRACKS
BAND 14
Co G 27
Co E 28
Co H 29
Co F 24
JOHNSON AVE
INFANTRY OFFICERS
LOGERLY PLACE
TO TARGET RANGE
MAYOR AVE
ENGINEER AND ARTILLERY OFFICERS
ENGINEER AND ARTILLERY BARRACKS
STABLES
GUN SHED
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7. The intravenous inoculation of filtered dengue blood into healthy men is followed by a typical attack of the disease.

8. The cause of the disease is, therefore, probably ultramicroscopic in size.

9. Dengue can be transmitted by the mosquito, *Culex fatigans*, and this is probably the most common method of its transmission.

10. The period of incubation in experimental dengue averages three days and fourteen hours.

11. Certain individuals are absolutely immune to dengue, as proven by our experiments.

12. Dengue is not a contagious disease, but is infectious in the same manner as is yellow fever and the malarial fevers.

In concluding this report we desire to express our appreciation of the encouragement and aid rendered us by Major-General Leonard Wood, commanding the Philippines Division, without which it would have been impossible for us to have made these researches. We also desire to thank Dr. Richard P. Strong, the director of the Biological Department of the Bureau of Science, for the use of apparatus, and Mr. Charles S. Banks, entomologist to the Bureau of Science, who rendered us assistance in the identification of mosquitoes.

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EXPLANATION OF MAP ATTACHED.

The map is intended to illustrate the spread of the epidemic of dengue at Fort William McKinley. The letters placed opposite the barracks indicate the company occupying them, and the figures accompaning the letters indicate the order in which the barracks were infected. The red line indicates the course of a small stream of water which formed an ideal breeding-place for mosquitoes, and which flowed near the barracks.

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